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Perfluorodecanoic Acid and Lipid Metabolism in the Rat

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Because PFDA treatment causes a dose-related reduction in feed intake, the response of vehicle-treated rats pair-fed to those receiving PFDA was monitored to distinguish direct effects of the perfluorinated fatty acid from those secondary to hypophagia. Carcass content of lipid phosphorus and free cholesterol decreased in dose-dependent fashion in both PFDA-treated and pair-fed rats. Carcass triacylglycerols diminished similarly, yet PFDA-treated rats at each dose had a higher concentration of neutral acylglycerols than vehicle-treated, pair-fed rats at the 80 mg/kg dose level, lopid phosphorus and free cholesterol as a proportion of carcass fat increased, whereas the share of the triacylglycerols declined. Because of the higher concentration of triacylglycerols in the carcass of rats treated with 80 mg/kg PFDA, enrichment of lipid phosphorus and free cholesterol in carcass fat was less than in their pair-fed partners. Lipid phosphorus and free cholesterol per hepatocyte are similar in both PFDA-treated rats and pair-fed partners. Liver triacylglycerols were markedly increased in PFDA treated rats. A similar but less extensive augmentary effect of PFDA on hepatic esterified cholesterol was found. Concentration of triacylglycerols in plasma was not elevated in OVER

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19. Cont

PFDA-treated rats, despite hepatic accumulation of esterified compounds. Free fatty acid and 3-hydroxybutyrate plasma level were similar in all treatment groups, including those receiving PFDA. Thus, the administration of PFDA appears to divert fatty acids from oxidation toward esterifcation in the liver.

ANNUAL TECHNICAL REPORT

AIR FORCE OFFICE OF SCIENTIFIC RESEARCH

TOXICOLOGY OF PERFLUORODECANOIC ACID

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SUMMARY

Studies on the effects of perfluorodecanoic acid (PFDA) on lipid metabolism in the rat after a single intraperitoneal dose (20, 40, 80 mg/kg) were completed. Because PFDA treatment causes a dose-related reduction in feed intake, the response of vehicle-treated rats pair-fed to those receiving PFDA was monitored to distinguish direct effects of the perfluorinated fatty acid from those secondary to hypophagia. A reduction in the carcass content of lipid phosphorus (phospholipid) and free cholesterol in rats treated with PFDA appeared to be consequent to hypophagia, as the pair-fed animals exhibited the same attenuation. Carcass triacylglycerols decreased in a dose-related fashion in both PFDA-treated rats and pair-fed partners. Yet, at every dose examined (20 - 80 mg/kg), PFDA-treated rats were found to have a higher concentration of triacylglycerols than their vehicle-treated, pair-fed counterparts. The amount of lipid phosphorus and free cholesterol per hepatocyte (per mg DNA) was similar in both PFDA-treated rats and their pair-fed controls. Liver triacylglycerols were markedly increased in a dose-related fashion in PFDA-treated rats, while being reduced in the vehicle-treated pair-fed rats. A similar but less extensive augmentary effect of PFDA on hepatic esterified cholesterol was found. Despite the hepatic accumulation of esterified compounds, concentration of triacylglycerols in the plasma was not elevated in the PFDA-treated rats. Therefore it appears PFDA treatment results in the diversion of fatty acids from oxidation towards esterification in the liver. This work has been completed and published in the journal "LIPIDS" (see enclosed reprints).

OBJECTIVES

The objectives of current and ongoing research on PFDA and its toxic effects in rats are:

1. To develop methodologies for the extraction, separation and quantitation of PFDA from biological samples.
2. To develop analogous methodologies for perfluorooctanoic acid (PFOA), a shorter-chain perfluorinated fatty acid similar to PFDA.
3. To validate the procedures for the extraction, separation and quantitation of the perfluorinated acids following in vivo administration of either PFDA or PFOA to rats.

STATUS OF THE RESEARCH

I. PFDA and Lipid Metabolism in the Rat.

The effects of PFDA (single ip dose 20, 40, or 80 mg/kg) on the composition of various lipid classes in carcass, plasma, and liver was examined 7 days after dosing. It was found that while the content of most lipid classes decreased as a result of the dose-dependent reduction in feed intake, carcass triacylglycerols were diminished to a lesser extent in PFDA-treated rats than in their pair-fed counterparts. In the liver, the content of phospholipid and free cholesterol was also affected as a result of PFDA-induced hypophagia, and these changes were paralleled in the vehicle-treated, pair-fed rats at each dose level examined. Hepatic content of triacyl-glycerols and esterified cholesterol was increased in a dose-related fashion in PFDA-treated rats, while being reduced in their pair-fed partners. No significant changes were observed in plasma lipids. Thus the administration of PFDA appears to divert fatty acids from oxidation towards esterification in the liver.

This work has been completed and has recently appeared as a publication in the journal "LIPIDS" (Van Rafelghem et al., 1988).

II. Analytical Methods.

We are currently working on the development and improvement of analytical methodologies for further study of the distribution and metabolism of PFDA as well as its effects on lipid metabolism in rats. The use of conventional lipid extraction and separation procedures has been found to lead to substantial loss of PFDA added to tissue. The electron-withdrawing fluorine atoms in the aliphatic chain of PFDA promote the dissociation of carboxylic hydrogen. Therefore, pH considerations are more critical with PFDA than with its nonperfluorinated analog, decanoic acid. The use of radiolabeled PFDA (synthesized in this laboratory) should aid further development of procedures for the extraction, separation and quantitation of this compound.

The behavior of PFOA during extraction, separation and isolation procedures will also be examined. Radiolabeled PFOA has been synthesized in this laboratory. In order to elucidate the metabolic fate and disposition of PFDA and PFOA, their behavior during extraction, separation and isolation must be known.

PUBLICATIONS

(Publications listed in previous annual reports are not included)

1. Van Rafelghem, M.J., Vanden Heuvel, J.P., Menahan, L.A., and Peterson, R.E. (1988). Perfluorodecanoic acid and lipid metabolism in the rat. Lipids 23, 671-678.

PERSONNEL

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INTERACTIONS

1. Preliminary results from this work were presented at the 28th Annual Meeting of the Society of Toxicology in Atlanta, GA, March, 1989.
2. Dr. Marc J. Van Rafelghem visited the Toxic Hazards Division at Wright-Patterson Air Force Base during the past year, in order to discuss research on PFDA in this laboratory with Dr. Mel Andersen and Ms. Marilyn George.

Perfluorodecanoic Acid and Lipid Metabolism in the Rat

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Alterations in lipid metabolism were examined in adult male Sprague-Dawley rats seven days after a single intraperitoneal injection of perfluorodecanoic acid (PFDA; 20, 40 or 80 mg/kg). Because PFDA treatment caused a dose-related reduction in feed intake, the response of vehicle-treated rats pair-fed to those receiving PFDA was monitored to distinguish direct effects of the perfluorinated fatty acid from those secondary to hypophagia. Carcass content of lipid phosphorus and free cholesterol decreased in dose-dependent fashion in both PFDA-treated and pair-fed rats. Carcass triacylglycerols diminished in a similar manner, yet PFDA-treated rats at each dose had a higher concentration of neutral acylglycerols than their vehicle-treated, pair-fed counterparts. In vehicle-treated, pair-fed rats at the 80 mg/kg dose level, lipid phosphorus and free cholesterol as a proportion of carcass fat increased, whereas the share of the triacylglycerols declined. Because of the higher concentration of triacylglycerols in the carcass of rats treated with 80 mg/kg PFDA, enrichment of lipid phosphorus and free cholesterol in carcass fat was less than in their pair-fed partners. The amount of lipid phosphorus and free cholesterol per hepatocyte was similar in both PFDA-treated rats and their pair-fed partners. Liver triacylglycerols were markedly increased in PFDA-treated rats. A similar but less extensive augmentary effect of PFDA on hepatic esterified cholesterol was found. Concentration of triacylglycerols in plasma was not elevated in PFDA-treated rats, in spite of hepatic accumulation of esterified compounds. Also, the plasma level of free fatty acids and 3-hydroxybutyrate was similar in all treatment groups, including those receiving PFDA. Thus, the administration of PFDA appears to divert fatty acids from oxidation toward esterification in the liver.
Lipids 23, 671-678 (1988).

Derivatives of perfluorosulfonic and perfluorocarboxylic acids have been used in a number of industrial applications as lubricants, plasticizers, wetting agents and corrosion inhibitors (1). Aqueous film-forming foams, used as fire extinguishants, contain mixtures of hydrocarbon and fluorocarbon surfactants (derivatized fatty acids) due to their superior surface-active properties (2).

Perfluorodecanoic acid (PFDA), representative of these perfluorinated fatty acids, resulted in a progressive reduction in feed intake, body weight loss, along with an increase in liver mass and changes in hepatic lipid composition in the rat (3,4). These effects followed treatment with a single intraperitoneal dose of PFDA. Recently, PFDA-treated rats were found to either gain less or lose more weight (depending on the dose administered) than vehicle-treated rats with the same caloric intake (5). Despite their lower body weight, PFDA-treated rats had a greater carcass fat content than their pair-fed counterparts (5).

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Abbreviations: PFDA, perfluorodecanoic acid; ANOVA, analysis of variance; SAS, statistical analysis system.

Lipid metabolism in the PFDA-treated adult male Sprague-Dawley rat has been examined in this study. By quantifying and comparing values for carcass, liver and plasma lipids in PFDA-treated and pair-fed animals, direct effects of PFDA on lipid metabolism were distinguished from those secondary to hypophagia.

MATERIALS AND METHODS

Chemicals. PFDA (also nonadecafluorodecanoic acid) was purchased from Aldrich Chemical Co. (Milwaukee, WI) and determined to have a purity of 87.4% by gas chromatography (6). All enzymes and cofactors were obtained from Boehringer Mannheim Corp. (Indianapolis, IN), with the exception of cholesterol oxidase and o-dianisidine, which were purchased from Sigma Chemical Co. (St. Louis, MO). Coomassie blue G-250 was obtained from Pierce Chemical Co. (Rockford, IL).

Animals. Male Sprague-Dawley rats (275-300 g), obtained from Harlan Sprague-Dawley (Indianapolis, IN), were individually housed in suspended stainless-steel cages in a temperature-controlled room (ca. 21 C) with a 12-hr light/dark cycle (lighted, 0500-1700 hr). A schedule was maintained so that ground feed (Purina Rat Chow, #5012, Ralston Purina Co., St. Louis, MO) was available from 1700 to 0830 hr and tap water was available ad libitum. An acclimation period of at least one wk was allowed before the initiation of an experiment. Rats then were paired on the basis of similar body weight. PFDA-treated rats received a single intraperitoneal injection of PFDA with dosing at 20, 40 or 80 mg/kg. At 24 hr after the PFDA-treated rat was dosed, its partner with a similar weight was given an equivalent volume of vehicle (propylene glycol/water, 50:50, v/v; 1 ml/kg). This vehicle-treated animal then received the same amount of feed its PFDA-treated partner had consumed during the previous 15-16 hr feeding period (pair-feeding). An additional group of vehicle-treated rats, with unlimited access to ground chow during the feeding period (15-16 hr), was included. Body weight and feed intake were measured daily for seven days following injection of PFDA or vehicle.

Experimental protocols. Two series of protocols were conducted. In the first set, carcass lipid was determined in rats killed by cervical dislocation seven days post-treatment between 1600 and 1700 hr. The gastrointestinal tract was removed, emptied of all contents and returned to the body cavity. Carcasses were weighed, placed in plastic bags, and frozen at −20 C. Each frozen carcass was ground to a homogeneous mixture with a Wiley laboratory mill cooled with liquid nitrogen. The ground carcasses were stored in individually sealed bags (Ziploc) at −20 C for later body composition analysis (5).

Liver and plasma lipid analyses were performed on tissue from rats exsanguinated by decapitation seven days post-treatment between 1300 and 1600 hr in the second series of protocols. Trunk blood was collected in a 50 ml disposable beaker containing 100 μl of 15% (w/v) potassium-ethylenediamine tetraacetic acid. Plasma was separated and stored at −70 C. A portion of liver was

frozen by freeze-stop technique (7) at the temperature of liquid nitrogen, weighed, and ground to a fine powder in a pre-cooled mortar and pestle. The frozen liver powder was stored in cryogenic vials under liquid nitrogen until the time of assay. The remaining liver tissue was excised and weighed. Total liver mass represents the combined weight of these two portions.

Analytical methods. Carcass, liver and plasma lipids were extracted with chloroform/methanol by a micro-adaptation of the procedure described originally by Folch et al. (8). Liver and carcass tissue powders, as well as plasma samples, were homogenized by mechanical disruption (2 × 15 sec with a 30-sec pause) in chloroform/methanol (2:1, v/v) in the proportion of 19 volumes of solvent to 1 volume of sample using a Tissumizer homogenizer with a micro probe (Tekmar Co., Cincinnati, OH). The crude extract was washed with 50 mM NaCl. Aliquots of the washed lipid extract were taken to dryness and analyzed for neutral glycerols (9). For lipid phosphorus determinations (10), digestion of lipid in a dried crude extract was accomplished by gentle refluxing with 70% perchloric acid in the presence of pure carborundum chips (133-A, Hengar and Co., Philadelphia, PA) on a micro-Kjeldahl digestion rack (11). Dipalmitoyl-dl-α-phosphatidylcholine, dipalmitoyl-dl-α-phosphatidylethanolamine, and sodium tribasic phosphate yielded equivalent standard curves. Determination of carcass fat (ether-extractable at 50 C) has been described (5).

Total cholesterol determination followed chemical hydrolysis of the cholesteryl esters to free cholesterol (12). Cholesterol then was oxidized enzymatically to cholest-4-en-3-one and hydrogen peroxide with cholesterol oxidase. The hydrogen peroxide generated was reacted with horseradish peroxidase in the presence of o-dianisidine to form a stable chromophore (13). Determination of free cholesterol was performed with the enzymatic reaction for cholesterol in the absence of chemical hydrolysis. Esterified cholesterol then was calculated as the difference between the values for total and free cholesterol. Enzymatic determination of 3-hydroxybutyrate was made on neutralized, perchloric acid extracts of plasma (14) as described by Williamson and Mellanby (15). The concentration of free fatty acids in plasma was quantitated by a sensitive micro method (16).

For DNA and protein analysis, liver homogenates in water (10%, w/v) were prepared by mechanical disruption (2 × 15 sec; Tissumizer) with a 30-sec pause between bursts with cooling in an ice-water mixture. After extraction of interfering lipids (17), DNA content was estimated by a micro-adaptation of the procedure described by Richards (18) using calf thymus DNA as standard. Total hepatic protein was determined by the dye-binding method of Bradford (19) with crystalline bovine serum albumin as standard.

Statistical analysis. The main effects of treatment (PFDA vs pair-fed) and dosing (20, 40 or 80 mg/kg) were analyzed by two-way analysis of variance (ANOVA) through the use of unweighted cell means (20). Significance of difference between PFDA-treated rats and their pair-fed counterparts at a given dose was detected by pair-wise comparison (20). Effect of dosing between treatment groups, PFDA or pair-fed, including comparison with the vehicle-treated group of rats with unlimited access to feed, was tested by one-way ANOVA and significance of differences between doses was analyzed using Scheffé's multiple comparison method (20). Linear function among doses was evaluated by testing for trends using orthogonal coefficients if effect of dosing in the one-way ANOVA was significant (21). The computations were performed with a VAX-750 computer using SAS (22). In all cases, significance was set at $p < 0.05$.

RESULTS

Body weight and feed intake. Cumulative feed intake of rats during the seven days following PFDA treatment was diminished in a dose-related manner with a remarkable decrease occurring at the 80 mg/kg dose (Fig. 1). Body weight of both PFDA-treated rats and their vehicle-treated, pair-fed counterparts at seven days post-treatment also was reduced in a dose-dependent fashion. Even though the cumulative feed intake of PFDA-treated rats and pair-fed counterparts was comparable at each dose level, the mean body weight of those receiving PFDA was lower at seven days after treatment (Fig. 1). When the change in body weight (calculated as the difference between the initial and final body weight) was analyzed by two-way ANOVA, the difference between PFDA-treated rats and their respective vehicle-treated, pair-fed partners was significant by pair-wise comparison at the 40 and 80 mg/kg dose levels.

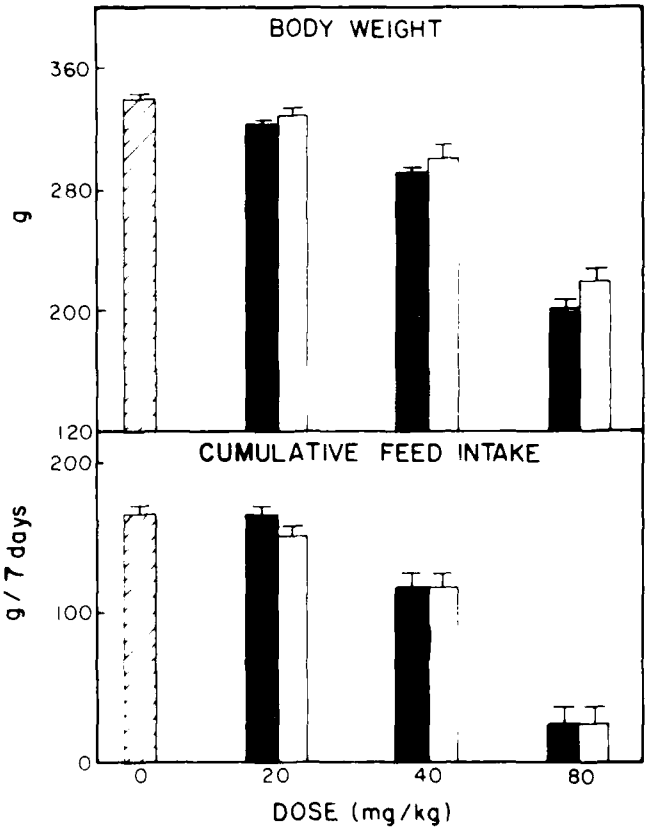


FIG. 1. Body weight and cumulative feed intake in PFDA- (20, 40 or 80 mg/kg) and vehicle-treated rats seven days after treatment. Vehicle-treated rats were either allowed unlimited access to feed (hatched bar) or were pair-fed (open bar) to PFDA-treated rats (closed bar). Each bar and its associated vertical line represent the mean and one S.E.M. (n = 4 for each treatment group).

Carcass. Content of triacylglycerols, whether expressed per g wet wt, carcass, or g fat, was significantly reduced in both PFDA-treated rats and their vehicle-treated, pair-fed counterparts at the 80 mg/kg dose level (Fig. 2). However, PFDA-treated rats at each dose had a higher concentration of triacylglycerols per g wet wt carcass than their pair-fed partners. A reduction in lipid phosphorus per carcass was found in the PFDA-treated rats as well as their vehicle-treated, pair-fed counterparts with increasing do[illegible] (Table 1). As a proportion of carcass fat, lipid phosphorus was enriched in the vehicle-treated, pair-fed rats at the 80 mg/kg dose level when compared with either the vehicle-treated group with unlimited access to feed or PFDA-treated rats at the same dose (Table 1). Lipid phosphorus per g fat was reduced in PFDA-treated rats at the 20 and 40 mg/kg dose levels but elevated at 80 mg/kg, in comparison with vehicle-treated rats with unlimited access to feed. With a dose of 40 mg/kg, the concentration of lipid phosphorus in carcass fat of rats receiving PFDA was less than their vehicle-treated, pair-fed partners and this difference was significant (Table 1). The concentration and content of free cholesterol in the carcass did not differ significantly between any of the treatment groups (Table 1). When expressed as a proportion of the carcass fat, free cholesterol was significantly higher in the vehicle-treated rats pair-fed to their PFDA-partners at the 80 mg/kg dose than in other vehicle-treated groups. A similar but less extensive increase was seen in the PFDA-treated rats at the 80 mg/kg dose, and the difference from their vehicle-treated pair-fed partners was significant (Table 1). An augmentary effect of PFDA on esterified cholesterol concentration and content in the carcass was demonstrated by a significant treatment effect in the two-way ANOVA; however, the difference between PFDA-treated rats and their vehicle-treated, pair-fed partners at each dose level was not significant by pairwise comparison (Table 1).

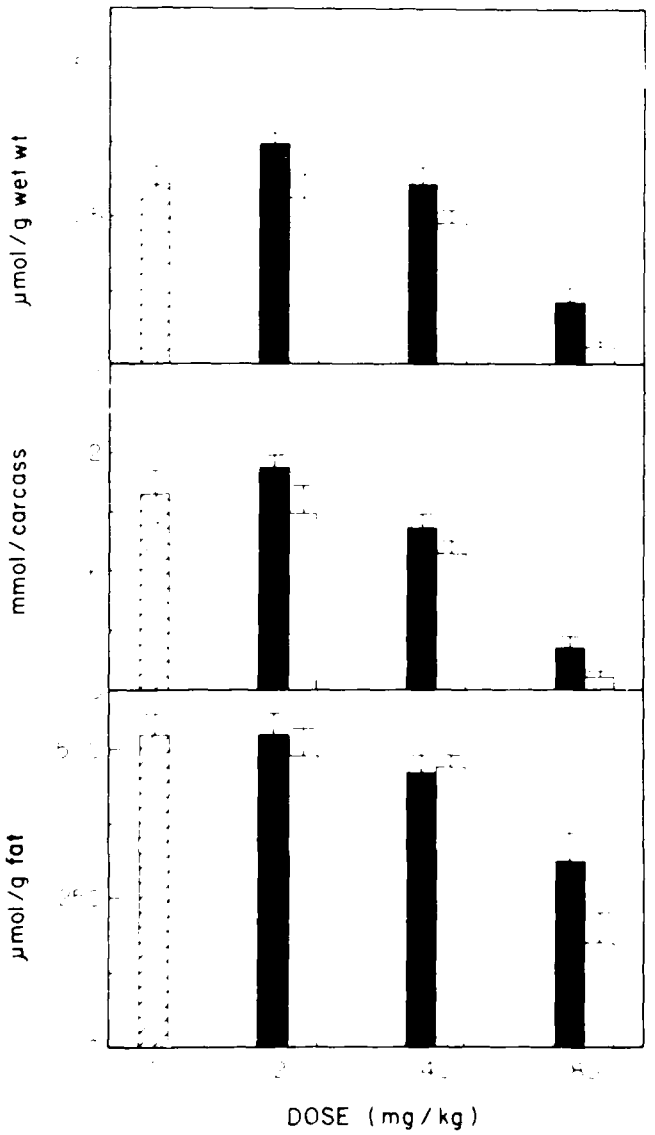


FIG. 2. Effect of PFDA treatment on carcass triacylglycerols in the rat. Vehicle-treated rats were either allowed unlimited access to feed (hatched bar) or were pair-fed (open bar) to PFDA-treated rats (closed bar). Each bar and its associated vertical line represent the mean and one S.E.M. (n = 4 for each treatment group).

Liver. Absolute and relative weight in PFDA-treated animals was significantly greater than in their vehicle-treated, pair-fed partners at all doses examined. When compared with the treatment groups receiving 20 and 40 mg/kg, the absolute increase in liver mass was significantly less, but relative liver weight was maintained in PFDA-treated rats at the 80 mg/kg dose level (Table 2). At the 80 mg/kg dose level, liver mass expressed on an absolute and relative basis, was reduced in vehicle-treated animals pair-fed to PFDA-treated rats (Table 2).

Hepatic DNA concentration was significantly higher in vehicle-treated rats pair-fed to those receiving 80 mg/kg PFDA when compared to the other vehicle-treated groups (Table 2). The concentration of DNA in the livers of PFDA-treated rats was lower than in their vehicle-treated pair-fed counterparts, and this difference was significant at the 20 and 80 mg/kg dose levels (Table 2). The DNA content per liver was reduced in a dose-related fashion in vehicle-treated rats, whereas it was maintained in the animals receiving PFDA at all doses. The difference in total DNA per liver between PFDA-treated rats and their vehicle-treated, pair-fed partners at the 40 and 80 mg/kg dose levels was significant (Table 2). Hepatic protein concentration was higher in vehicle-treated, pair-fed groups than in their PFDA-treated partners, and this difference was significant at the 80 mg/kg dose level (Table 2). At the 80 mg/kg dose level, total protein per liver was reduced significantly in rats receiving PFDA as well as their vehicle-treated, pair-fed partners. When hepatic protein was expressed per mg DNA, no statistically significant differences were detected between any of the treatment groups (Table 2).

Hepatic concentration of lipid phosphorus and free cholesterol was similar in all treatment groups (Table 3). When expressed per liver, a significant reduction in the content of lipid phosphorus and free cholesterol was found in vehicle-treated, pair-fed rats at the 80 mg/kg dose level, when compared with either the other vehicle-treated groups or those receiving PFDA. On a cellular basis (per mg DNA), lipid phosphorus but not free cholesterol was influenced significantly by dose with a decline at the 80 mg/kg dose level (Table 3).

TABLE 1

Effects of PFDA on Carcass Lipid Composition of Adult Male Rats[a]

Dose mg kg	Treatment	Lipid phosphorus			Cholesterol					
					Free			Esterified		
		μmol g wet wt	mmol carcass	μmol g fat	μmol g wet wt	mmol carcass	μmol g fat	μmol g wet wt	mmol carcass	μmol g fat
0	Unlimited-fed	10.1 ± 0.6^{e}	3.26 ± 0.16^{b,e}	179 ± 25^{b,c,e}	3.78 ± 1.21	1.19 ± 0.36	70.1 ± 31.0^{b,c,e}	0.75 ± 0.19	0.24 ± 0.06	13.3 ± 4.0
20	PFDA	8.95 ± 0.54	2.72 ± 0.15^{b,e}	127 ± 8^{b}	2.91 ± 0.12	0.89 ± 0.03	41.3 ± 2.1^{b}	2.07 ± 0.54	0.63 ± 0.17	29.3 ± 7.7
	Pair-fed	8.14 ± 0.38^{e,f}	2.50 ± 0.11^{f}	143 ± 11^{e}	3.05 ± 0.10	0.93 ± 0.01	53.6 ± 4.5^{e}	0.81 ± 0.16	0.25 ± 0.05	14.1 ± 2.5
40	PFDA	8.03 ± 0.79	2.17 ± 0.22^{c,d}	118 ± 13^{b,*}	3.02 ± 0.07	0.82 ± 0.03	45.9 ± 2.4^{b,c}	1.18 ± 0.28	0.32 ± 0.08	17.9 ± 4.3
	Pair-fed	8.25 ± 0.54^{e,f}	2.33 ± 0.19^{f}	161 ± 8^{e}	3.09 ± 0.18	0.85 ± 0.07	59.2 ± 5.1^{e}	0.86 ± 0.20	0.25 ± 0.06	17.0 ± 4.1
80	PFDA	8.89 ± 0.43	1.70 ± 0.08^{d}	283 ± 51^{c}	3.55 ± 0.27	0.68 ± 0.06	110 ± 16^{c,*}	1.17 ± 0.60	0.32 ± 0.11	60.7 ± 26.4
	Pair-fed	7.37 ± 0.63^{f}	1.53 ± 0.13^{g}	413 ± 14^{f}	3.46 ± 0.24	0.72 ± 0.04	194 ± 10^{f}	0.82 ± 0.28	0.17 ± 0.07	49.0 ± 18.0

[a]Values are reported as the mean and S.E.M. for three to four rats.

Mean values in a column not followed by the same superscript (b, c, d) are significantly different from other dose levels within the PFDA treatment group using Scheffe's multiple comparison method ($p < 0.05$). The unlimited-fed group serves as the zero-dose level.

Mean values in a column not followed by the same superscript (e, f, g) are significantly different from other dose levels within the pair-fed treatment group using Scheffe's multiple comparison method ($p < 0.05$). The unlimited-fed group serves as the zero-dose level.

*Difference from the respective pair-fed group is significant ($p < 0.05$).

TABLE 2

Liver Mass and Hepatic Content of DNA and Protein in Male Rats Seven Days Following PFDA Treatment[a]

Dose mg kg	Treatment	Liver mass		DNA		Protein		
		Absolute g wet wt	Relative % body wt	mg/g wet wt	mg/liver	mg g wet wt	g liver	mg mg DNA
0	Unlimited-fed	13.0 ± 0.3^{b,c,d}	3.78 ± 0.04^{b,d}	2.12 ± 0.09^{b,c,d}	27.4 ± 1.3^{d}	134 ± 13	1.74 ± 0.20	64 ± 8
20	PFDA	15.7 ± 0.5^{b,*}	4.69 ± 0.07^{c,*}	1.92 ± 0.04^{b,*}	30.2 ± 0.8	113 ± 4	1.78 ± 0.10	59 ± 2
	Pair-fed	12.2 ± 0.2^{d}	3.59 ± 0.07^{d}	2.20 ± 0.10^{d}	26.8 ± 1.3^{d}	137 ± 21	1.68 ± 0.27	64 ± 12
40	PFDA	15.3 ± 0.3^{b,*}	5.09 ± 0.07^{c,*}	1.98 ± 0.09^{b}	30.3 ± 1.3*	118 ± 9	1.80 ± 0.14	60 ± 7
	Pair-fed	11.5 ± 1.1^{d}	3.54 ± 0.28^{d}	2.27 ± 0.16^{d}	25.6 ± 0.8^{d,e}	135 ± 16	1.58 ± 0.28	62 ± 10
80	PFDA	12.3 ± 1.1^{c,*}	5.17 ± 0.20^{c,*}	2.40 ± 0.11^{c,*}	29.4 ± 2.1*	101 ± 2*	1.24 ± 0.10	42 ± 1
	Pair-fed	5.6 ± 1.1^{e}	2.23 ± 0.31^{e}	3.00 ± 0.17^{e}	16.9 ± 3.6^{e}	158 ± 13	0.91 ± 0.24	53 ± 3

[a]Values are reported as the mean and S.E.M. for four rats.

Mean values in a column not followed by the same superscript (b, c) are significantly different from other dose levels within the PFDA treatment group using Scheffé's multiple comparison method ($p < 0.05$). The unlimited-fed group serves as the zero-dose level.

Mean values in a column not followed by the same superscript (d, e) are significantly different from other dose levels within the pair-fed treatment group using Scheffé's multiple comparison method ($p < 0.05$). The unlimited-fed group serves as the zero-dose level.

*Difference from the respective pair-fed group is significant ($p < 0.05$).

TABLE 3

Liver Lipid Phosphorus and Free Cholesterol in Male Rats Seven Days After PFDA Treatment[a]

Dose mg/kg	Treatment	Lipid phosphorus			Free cholesterol		
		μmol/g wet wt	μmol/liver	μmol/mg DNA	μmol/g wet wt	μmol/liver	μmol/mg DNA
0	Unlimited-fed	29.1 ± 1.3	377 ± 23[b]	13.9 ± 1.0	3.10 ± 0.15	39.9 ± 1.8[b]	1.46 ± 0.05
20	PFDA	33.9 ± 5.4	527 ± 77	17.6 ± 2.7	3.21 ± 0.43	51.5 ± 5.9	1.49 ± 0.14
	Pair-fed	31.7 ± 1.6	392 ± 27[b]	14.6 ± 1.2	3.39 ± 0.16	40.9 ± 1.0[b]	1.51 ± 0.14
40	PFDA	28.2 ± 1.4	431 ± 16	14.3 ± 0.8	2.81 ± 0.23	43.8 ± 4.0	1.46 ± 0.04
	Pair-fed	35.4 ± 3.6	417 ± 83[b]	16.1 ± 2.8	3.03 ± 0.12	35.0 ± 4.1[b,c]	1.36 ± 0.12
80	PFDA	28.2 ± 0.4	347 ± 31*	11.8 ± 0.4	4.10 ± 0.74	49.7 ± 7.9*	1.71 ± 0.29
	Pair-fed	27.4 ± 2.1	152 ± 30[c]	9.2 ± 0.8	3.97 ± 0.44	21.9 ± 4.6[c]	1.34 ± 0.17

[a]Values are reported as the mean and S.E.M. for three to four rats.

Mean values in a column not followed by the same superscript (b, c) are significantly different from other dose levels in the pair-fed treatment group using Scheffé's multiple comparison method ($p < 0.05$). The unlimited-fed group serves as the zero-dose level.

*Difference from the respective pair-fed group is significant ($p < 0.05$).

TABLE 4

Plasma Lipid Composition of Male Rats Seven Days Following PFDA Treatment[a]

Dose mg/kg	Treatment	Free fatty acids	3-Hydroxybutyrate	Triacylglycerols	Lipid phosphorus	Cholesterol	
						Free	Esterified
0	Unlimited-fed	0.29 ± 0.03	0.10 ± 0.01	0.33 ± 0.06[b,c]	1.27 ± 0.08[b,e]	0.45 ± 0.06	1.18 ± 0.19[e]
20	PFDA	0.41 ± 0.05	0.14 ± 0.03	0.21 ± 0.03[b,c]	0.91 ± 0.04[c]	0.37 ± 0.02*	0.62 ± 0.09*
	Pair-fed	0.30 ± 0.02	0.17 ± 0.04	0.28 ± 0.03	1.08 ± 0.04[e]	0.46 ± 0.02	0.88 ± 0.05[e,f]
40	PFDA	0.32 ± 0.06	0.08 ± 0.03	0.37 ± 0.03[b]	0.97 ± 0.02[c]	0.41 ± 0.05	0.56 ± 0.06*
	Pair-fed	0.36 ± 0.07	0.23 ± 0.09	0.23 ± 0.06	0.93 ± 0.08[e,f]	0.43 ± 0.03	0.94 ± 0.03[e,f]
80	PFDA	0.31 ± 0.01	0.21 ± 0.09	0.17 ± 0.03[c]	0.51 ± 0.02[d]	0.48 ± 0.07*	0.73 ± 0.14
	Pair-fed	0.24 ± 0.08	0.17 ± 0.07	0.17 ± 0.04	0.58 ± 0.12[f]	0.30 ± 0.03	0.54 ± 0.08[f]

[a]Values are reported as the mean and S.E.M. for three to four rats and are expressed as μmol/ml plasma.

Mean values in a column not followed by the same superscript (b, c, d) are significantly different from other dose levels within the PFDA treatment group using Scheffé's multiple comparison method ($p < 0.05$). The unlimited-fed group serves as the zero-dose level.

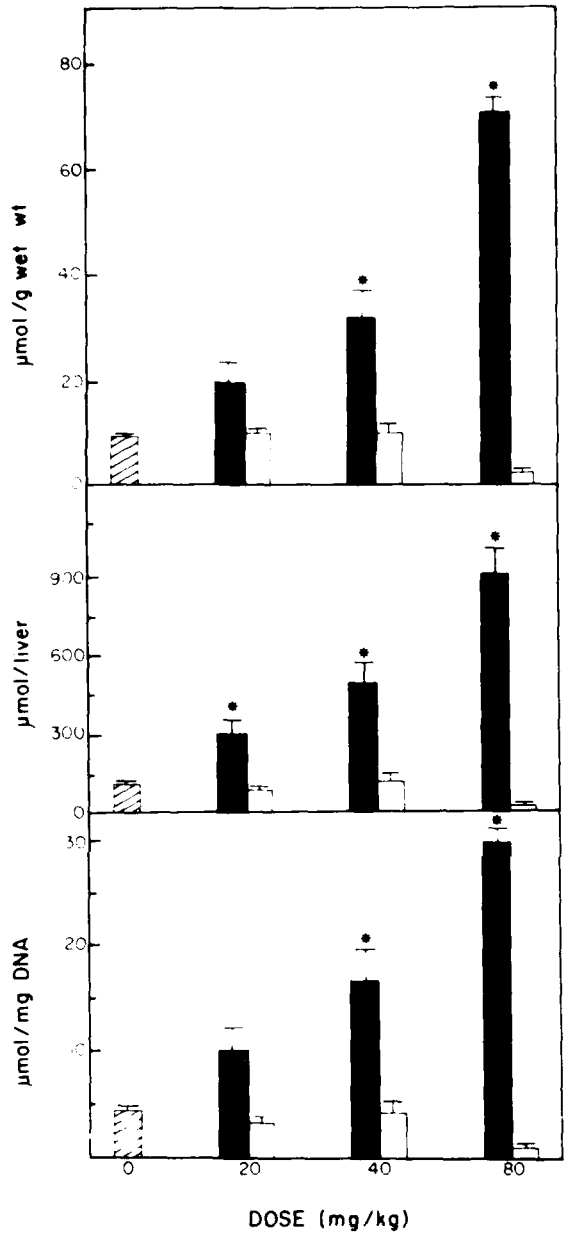
Mean values in a column not followed by the same superscript (e, f, g) are significantly different from other dose levels within the pair-fed treatment group using Scheffé's multiple comparison method ($p < 0.05$). The unlimited-fed group serves as the zero-dose level.

*Difference from the respective pair-fed group is significant ($p < 0.05$).

PFDA treatment resulted in a dose-related increase in liver triacylglycerols (Fig. 3). When expressed per g wet wt or mg DNA, the increment of hepatic triacylglycerols between PFDA-treated rats and their vehicle-treated, pair-fed counterparts at the 40 and 80 mg/kg dose levels was significant (Fig. 3). The content of triacylglycerols per liver was significantly greater in PFDA-treated rats than their vehicle-treated, pair-fed partners at all doses examined. However, liver triacylglycerols expressed per g wet wt, liver or mg DNA, were diminished in the vehicle-treated rats pair-fed to those receiving 80 mg/kg PFDA when compared with the other vehicle-treated groups (Fig. 3).

The response of hepatic esterified cholesterol to PFDA treatment was similar but less pronounced than that of liver triacylglycerols. A dose-related increase in liver esterified cholesterol was found in PFDA-treated rats (Fig. 4). The difference in hepatic esterified cholesterol between rats receiving PFDA and their pair-fed partners was significant at the 80 mg/kg dose level, whether expressed per g wet wt, liver or mg DNA. Hepatic esterified cholesterol also was elevated in PFDA-treated rats at the 40 mg/kg dose, but the difference from their vehicle-treated, pair-fed partners was statistically significant only when expressed per liver.

Plasma. The plasma level of free fatty acids and 3-hydroxybutyrate was similar in all treatment groups (Table 4). The concentration of triacylglycerols and lipid phosphorus in plasma decreased in a dose-related fashion in both PFDA-treated and pair-fed groups, but a significant difference between treatments was not detected by two-way ANOVA (Table 4). With a dose of 20 mg/kg, the plasma concentration of free cholesterol was lower in rats receiving PFDA than in their vehicle-treated, pair-fed partners (Table 4). At the 80 mg/kg dose level, the concentration of free cholesterol was higher in the plasma of PFDA-treated rats than their pair-fed counterparts, and the difference was significant. This dissimilarity resulted from a decrease in the plasma level of free cholesterol in the vehicle-treated, pair-fed group (Table 4). Also, the vehicle-treated rats pair-fed to their partners receiving 80 mg/kg PFDA had a concentration of



FIC. 3. Liver triacylglycerols in rats seven days after PFDA treatment. Vehicle-treated rats were either allowed unlimited access to feed (hatched bar) or were pair-fed (open bar) to PFDA-treated rats (closed bar). Each bar and its associated vertical line represent the mean and one S.E.M. (n = 4 for each treatment group). *Difference from the respective pair-fed group is significant ($p < 0.05$).

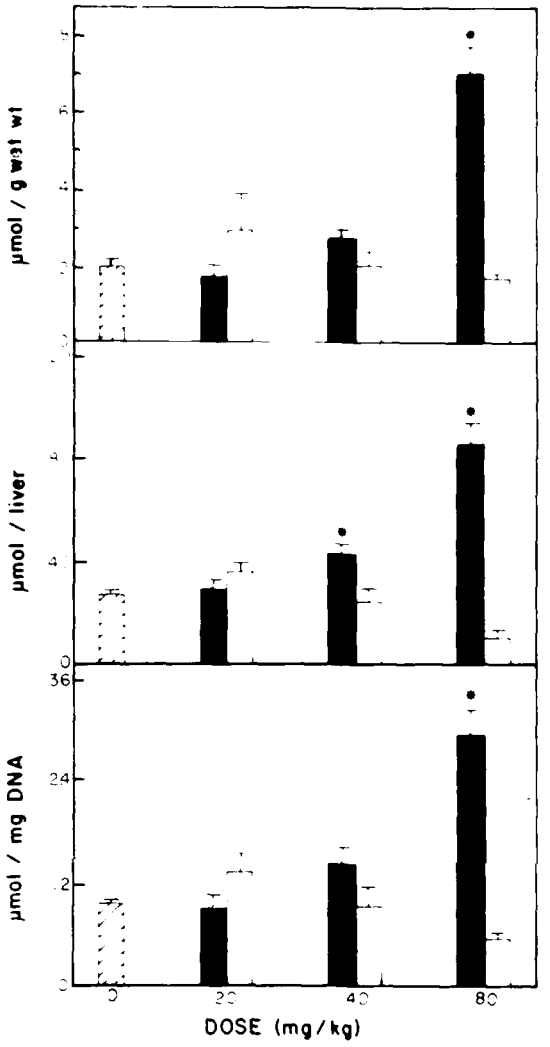


FIG. 4. Liver esterified cholesterol in rats seven days after PFDA treatment. Vehicle-treated rats were either allowed unlimited access to feed (hatched bar) or were pair-fed (open bar) to PFDA-treated rats (closed bar). Each bar and its associated vertical line represent the mean and one S.E.M. (n = 4 for each treatment group). *Difference from the respective pair-fed group is significant ($p < 0.05$).

esterified cholesterol in plasma that was lower than the other vehicle-treated groups, whether pair-fed or with unlimited access to feed (Table 4). In PFDA-treated rats, regardless of dose, the plasma level of esterified cholesterol was lower than that of the vehicle-treated group with unlimited access to feed (Table 4). At the 20 and 40 mg/kg dose levels, the concentration of esterified cholesterol in plasma also was significantly lower in PFDA-treated rats than their vehicle-treated, pair-fed partners.

DISCUSSION

Following a single intraperitoneal injection of PFDA, alterations in lipid metabolism of the adult male Sprague-Dawley rat were investigated seven days after dosing. Because the administration of PFDA causes a dose-dependent reduction in feed (caloric) intake (3-5), it was important to take this into account in the interpretation of the data through the use of a vehicle-treated rat, pair-fed to each one receiving PFDA. Thus, the primary effects of PFDA on lipid metabolism could be distinguished both qualitatively and quantitatively from those secondary to hypophagia.

A dose-related decrease in both body and carcass weight, associated with a diminution in caloric intake, was seen in rats receiving PFDA as well as their vehicle treated, pair-fed counterparts. Reduction in the carcass content of lipid phosphorus (phospholipid) and free

cholesterol in rats treated with PFDA would seem to be consequent to hypophagia and resulting weight loss, as the appropriate vehicle-treated, pair-fed animals exhibited the same attenuation. Carcass triacylglycerols decreased in a dose-related fashion in both PFDA-treated rats and their pair-fed partners, due to the negative caloric balance, i.e., feed intake. Yet, at every dose level examined (20-80 mg/kg), PFDA-treated rats were found to have a concentration of triacylglycerols in their carcass greater than that of their vehicle-treated, pair-fed counterparts. A similar increment in carcass ether-extractable fat also was detected between PFDA- and vehicle-treated rats (5). Differences in carcass lipid composition between the various treatment groups also were compared by expressing each lipid class as a proportion of ether-extractable fat. At the highest dose (80 mg/kg) examined, the loss of ether-extractable fat in the vehicle-treated rats pair-fed to their partners receiving PFDA was accompanied by an elevation in the proportion of lipid phosphorus and free cholesterol with a concomitant reduction in triacylglycerols. In rats treated with 80 mg/kg PFDA, a similar pattern but less extensive shift in the composition of the ether-extractable fat was found. This was the result of the higher concentration of triacylglycerols found in the carcasses of PFDA-treated rats than their pair-fed partners.

Major shifts in the relative percentages of fatty acids (3) and an increase in the activity of peroxisomal fatty acyl-CoA oxidase (25,26) in the rat liver, following the administration of PFDA would suggest that the perfluorinated fatty acid probably interacts with hepatic lipid metabolism. So that the changes in the concentrations of the various lipids could be compared, differences in hepatic cellularity between the different treatment groups had to be considered. In PFDA-treated rats, liver mass (absolute and relative) increased when compared with that of appropriate vehicle-treated, pair-fed animals. At each dose examined (20-80 mg/kg), an increase in DNA concentration (indicative of a smaller hepatocyte) in vehicle-treated, pair-fed rats was detected when contrasted with their partners receiving PFDA. Reduction in the DNA content per liver (indicating a loss of cells) also was detected in vehicle-treated, pair-fed rats at the 40 and 80 mg/kg dose levels, seven days post-treatment. Content of phospholipid and free cholesterol per total liver was reduced in the vehicle-treated rats pair-fed to those receiving 80 mg/kg PFDA. Corresponding decreases were reported in feed-restricted animals (27-29). A similar decrease in liver content of phospholipids and free cholesterol was not found in the PFDA-treated rats. In spite of this apparent difference between the PFDA-treated animals and their vehicle-treated, pair-fed counterparts, phospholipid and free cholesterol per cell was not different among the various treatment groups when changes in cellularity were taken into account.

Even though there was no effect of PFDA on the amount of phospholipid and free cholesterol per cell, a marked increase in the content of triacylglycerols per liver was detected. The accumulation of hepatic triacylglycerols can occur for a number of reasons, including increased fatty acid load (endogenous and exogenous), decreased fatty acid oxidation, increased synthesis and/or reduced breakdown of triacylglycerols, and a decreased export as very low density lipoproteins (30). Regulation of triacylglycerol synthesis is complex and not fully understood. However, the availability of fatty acids plays a major role in the esterification pathway (31). Free fatty acids in the circulation are taken up by the liver in a concentration-dependent manner (32,33). The level of free fatty acids in the plasma of PFDA-treated rats was similar to those receiving vehicle, whether pair-fed or with unlimited access to feed. However, this determination was an indication of the concentration of free fatty acids at a single time point, seven days after dosing. The increase in the relative percentage of oleic acid in the lipids of livers from PFDA-treated rats (3,4) would suggest that the peripheral fat stores (34) were the source of long chain fatty acyl-CoA's for the synthesis of triacylglycerols. Augmentation in the hepatic content of triacylglycerols already was detected at the lowest dose (20 mg/kg) of PFDA examined. It is possible that PFDA, similar to other carboxylic acids such as 2-bromooctanoic acid and 4-pentenoic acid (35-38), does inhibit fatty acid oxidation, thereby diverting fatty acids from oxidation toward esterification. As with triacylglycerols, a similar but less pronounced augmentation in the content of esterified cholesterol was detected in the PFDA-treated rats. While cholesterol ester synthesis appears to depend on the supply of unesterified cholesterol, moderate increases in long chain fatty acyl-CoA's also can stimulate this esterification pathway (39). Thus, it is conceivable that in PFDA-treated rats this pathway becomes available for the removal of excess long chain fatty acyl-CoA's in addition to esterification as triacylglycerols. However, synthesis of triacylglycerols would appear to be much more sensitive to increases in the concentration of long chain fatty acyl-CoA's. The hepatic capacity for formation of triacylglycerols would seem to be much greater than cholesterol esters.

PFDA has been found to be very persistent and can be detected in the liver of male Fischer-344 rats 30 days after a single intraperitoneal dose of 50 mg/kg (40). If PFDA and/or its activated derivatives (CoA or carnitine esters) were able to partially inhibit hepatic oxidation of fatty acids (35-38), one would expect triacylglycerols to increase. Thus, the impairment of fatty acid oxidation by PFDA could then result in a diminished NADH supply for ATP generation. This has been found in the presence of bromooctanoate, a known inhibitor of fatty acid oxidation (41). The resultant lowering of hepatic ATP concentration could in turn lead to a decrease in protein synthesis including that of apolipoproteins, e.g., apo-B needed for export of triacylglycerols from the liver (30,42). Even though hepatic DNA, phospholipid and free cholesterol content were maintained in PFDA-treated rats at 80 mg/kg, the amount of protein per hepatocyte was decreased. The increase in the activities of hepatic malic enzyme and L-glycerol-6-phosphate dehydrogenase found at lower doses of PFDA (20 and 40 mg/kg) was reduced or obliterated, respectively, in rats receiving 80 mg/kg (43). Thus, a potential decreased hepatic synthesis of apolipoproteins limiting very low density lipoprotein synthesis could contribute to the accumulation of triacylglycerols found in PFDA-treated rats at the highest dose examined (80 mg/kg). Furthermore, plasma triacylglycerols were not elevated in PFDA-treated rats, despite the hepatic augmentation of esterified compounds. The increase in hepatic content of triacylglycerols and esterified

cholesterol would suggest diversion of long chain fatty acyl-CoA's from oxidation toward esterification in livers of PFDA-treated rats.

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REFERENCES

1. Guenthner, R.A., and Vietor, L.M. (1962) *Ind. Eng. Chem. Prod. Res. Dev. 1*, 165-169.
2. Shinoda, K., and Nomura, T. (1980) *J. Phys. Chem. 84*, 365-369.
3. Olson, C.T., and Andersen, M.E. (1983) *Toxicol. Appl. Pharmacol. 70*, 362-372.
4. George, M.E., and Andersen, M.E. (1986) *Toxicol. Appl. Pharmacol. 85*, 169-180.
5. Van Rafelghem, M.J., Noren, C.W., Menahan, L.A., and Peterson, R.E., *Toxicol. Lett. 40*, 57-69.
6. Kelling, C.K., Van Rafelghem, M.J., Drake, R.L., Menahan, L.A., and Peterson, R.E. (1986) *J. Biochem. Toxicol. 1*(3), 23-37.
7. Wollenberger, A., Ristau, O., and Schoffa, G. (1960) *Pflugers Arch. 270*, 399-412.
8. Folch, J., Lees, M., and Sloane Stanley, G.H. (1957) *J. Biol. Chem. 226*, 497-509.
9. Laurell, S. (1966) *Scand. J. Clin. Lab. Invest. 18*, 668-672.
10. Christie, W.W. (1973) in *Lipid Analysis*, pp. 222-223, Pergamon Press, New York.
11. Zilversmit, D.B., and Davis, A.K. (1950) *J. Lab. Clin. Med. 35*, 155-160.
12. Deacon, A.C., and Dawson, P.J.G. (1979) *Clin. Chem. 25*, 976-984.
13. Salè, F.O., Marchesini, S., Fishman, P.H., and Berra, B. (1984) *Anal. Biochem. 142*, 347-350.
14. Mellanby, J., and Williamson, D.H. (1974) in *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.) pp. 1840-1843, Academic Press, New York.
15. Williamson, D.H., and Mellanby, J. (1974) in *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.) pp. 1836-1839, Academic Press, New York.
16. Hron, T.W., and Menahan, L.A. (1981) *J. Lipid Res. 22*, 377-381.
17. Livingston, J.N., Cuatrecasas, P., and Lockwood, D.H. (1974) *J. Lipid Res. 15*, 26-32.
18. Richards, G.M. (1974) *Anal. Biochem. 57*, 369-376.
19. Bradford, M.M. (1976) *Anal. Biochem. 72*, 248-254.
20. Milliken, G.A., and Johnson, D.E. (1984) in *Analysis of Messy Data. Volume I: Designed Experiments*, pp. 35-36, 146-155, Van Nostrand Reinhold Co., New York.
21. Neter, J., and Wasserman, W. (1974) *Applied Linear Statistical Methods*, Richard D. Irwin, Homewood, IL.
22. SAS User's Guide: Statistics (1985) SAS Institute Inc., Cary, NC.
23. Owen, J.S., and McIntyre, N. (1982) *Trends Biochem. Sci. 7*, 95-98.
24. Hollanders, B., Mougin, A., N'Diaye, F., Hentz, E., Aude, X., and Girard, A. (1986) *Comp. Biochem. Physiol. 84B*, 83-98.
25. Van Rafelghem, M.J., Andersen, M.E., Lane, S., Luking, S., and Harrison, E.H. (1984) *Toxicologist 4*, 174.
26. Harrison, E.H., Lane, J.S., Luking, S., Van Rafelghem, M.J., and Andersen, M.E. (1988) *Lipids 23*, 115-119.
27. Freedland, R.A. (1967) *J. Nutr. 91*, 489-495.
28. Herrera, E., and Freinkel (1968) *Biochim. Biophys. Acta 170*, 244-253.
29. Weigand, W., Hannappel, E., and Brand, K. (1980) *J. Nutr. 110*, 669-674.
30. Dianzani, M.U. (1979) in *Toxic Injury of the Liver. Part A* (Farber, E., and Fisher, M.M., eds.) pp. 281-331, Marcel Dekker, Inc., New York.
31. Zammit, V.A. (1984) *Prog. Lipid Res. 23*, 39-67.
32. Van Harken, D.R., Dixon, C.W., and Heimberg, M. (1969) *J. Biol. Chem. 224*, 2278-2285.
33. Goreski, C.A., Daly, D.S., Mishkin, S., and Arias, I.M. (1978) *Am. J. Physiol. 234*(6), E542-E553.
34. Kohout, M., Braun, T., and Mihalec, C. (1965) *Physiol. Bohemoslov. 14*, 460-465.
35. Raaka, B.M., and Lowenstein, J.M. (1979) *J. Biol. Chem. 254*, 3303-3310.
36. Raaka, B.M., and Lowenstein, J.M. (1979) *J. Biol. Chem. 254*, 6755-6762.
37. Osmundsen, H., and Sherratt, H.S.A. (1978) *Biochem. Soc. Trans. 6*, 84-88.
38. Schulz, H. (1987) *Life Sci. 40*, 1443-1449.
39. Spector, A.A., Mathur, S.N., and Kaduce, T.L. (1979) *Prog. Lipid Res. 18*, 31-53.
40. George, M.E., and Andersen, M.E. (1986) *Toxicologist 6*, 315.
41. Danis, M., Kauffman, F.C., Evans, R.K., and Thurman, R.G. (1981) *J. Pharmacol. Exp. Ther. 219*, 383-388.
42. Lombardi, B. (1965) *Fed. Proc. 24*, 1200-1205.
43. Kelling, C.K., Van Rafelghem, M.J., Menahan, L.A., and Peterson, R.E. (1987) *Biochem. Pharmacol. 36*, 1337-1344.

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